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METABOLIC FACTORS INFLUENCING MYOCARDIAL RECOVERY FROM ACIDOSIS (CIC3)

FINAL REPORT

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13. ABSTRACT *(Maximum 200 words)*

The mechanisms involved in cardiac dysfunction during acidosis were explored in an isolated heart model. Metabolic acidosis was observed to cause marked functional and energy metabolic derangement consistent with a primary impairment of energy production. Synergism with hypoxia was also demonstrated in this model. The site of metabolic blockage during acidosis was observed to be at the level of oxidative metabolism and not glycolysis. Isolated cardiac mitochondrial studies, however, did not demonstrate any direct effect of acidosis on mitochondrial respiration or coupling. Studies with respiratory acidosis were complicated by the coexistence of relative hypoxia which affected the model deleteriously. Treatment of acidotic isolated hearts with the experimental buffer, Carbicarb, caused marked increases in intracellular pH as well as functional and metabolic improvements. Isotonic rather than hypertonic Carbicarb was found to be substantially more effective in this model. The superiority of isotonic to hypertonic Carbicarb may be related to the tendency of hypertonic but not isotonic Carbicarb to significantly increase cytosolic sodium concentrations.

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FOREWORD

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INTRODUCTION

The clinical treatment of acute systemic acidosis is currently extremely controversial (1,2). Although it appears that systemic acidosis is functionally deleterious to the function of different organs, the mechanisms of these effects are still unclear. Recent work in the heart has defined that most of the impaired contractile function of the heart during metabolic acidosis is related to decreased contractile response (3,5) to a normal or increased cytosolic calcium concentration (6,7). However, it is not clear from existing studies what biochemical factors mediate this impaired contractile response. Intracellular acidosis is likely to be one such factor (3) although it recent studies from our laboratory suggest a synergistic effect of increases in inorganic phosphate (Pi) (5,8). The molecular basis of this is synergism may be related to alteration of actin-myosin cross-bridge cycling by the monovalent form of inorganic phosphate (Pim), the concentration of which is determined by the tissue concentration of Pi and pHi (9,10).

On this background, considerable concern exists regarding the clinical utility of sodium bicarbonate, the most commonly employed agent for acute alkalinization in treating systemic acidosis (1). Experimental work suggests that sodium bicarbonate is a relatively poor alkalinizer of the extracellular fluid, and because of increases in PCO_2 may have a paradoxical effect on pHi. This has perhaps best been shown by Arieff and coworkers who found that treating organic acidosis in dogs with bicarbonate had deleterious hemodynamic effects and caused a paradoxical decrease in liver pH as measured by DMO distribution (11,12). In a recent controlled clinical study, bicarbonate did not improve hemodynamics in critically ill patients with lactic acidosis (13). Carbicarb is a recently formulated buffer which may have advantages over sodium bicarbonate. This buffer is made up of 1/3M sodium bicarbonate and 1/3 M disodium carbonate and thus is equal in sodium concentration to clinically employed (1M) sodium bicarbonate (14). Because the carbonate ion is rapidly converted *in vivo* to form two bicarbonate ions (consuming CO_2 in the process), the effect of Carbicarb on the serum bicarbonate concentration is essentially the same as that of equal volumes of 1M sodium bicarbonate (14-16). The CO_2 tension of Carbicarb, however, is significantly lower than the CO_2 tension of sodium bicarbonate, and the net effect of Carbicarb (i.e. the sum of the CO_2 consumption by the carbonate component and some generation of CO_2 by the bicarbonate component) is to consume CO_2 *in vitro* and *in vivo* . Moreover, Carbicarb is better tolerated hemodynamically *in vivo* than sodium bicarbonate, and is an effective alkalinizer of the intracellular space (8,17-20).

Virtually all forms of impaired tissue oxygenation or ventilation are associated with significant acidosis which further impairs the physiologic function of that organ (21-25). Impaired tissue ventilation and oxygenation commonly complicate battlefield injuries; therefore, the therapy of acidosis is of particular relevance to care of the soldier wounded on the battlefield. As effective therapy of this acidosis would best be administered as soon as possible, the site for such administration would be the battlefield. Therefore, the study of acidosis and its therapy is directly relevant to battlefield injuries and the USAMRDC mission.

During the past several years, the applicant's laboratory has focused its efforts on the study of acid-base disorders. In our earlier studies, we employed *in vivo* NMR methods to monitor intracellular brain pH and liver pH in different models of systemic acidosis and during alkalinization therapy with bicarbonate or Carbicarb. In these studies, bicarbonate was found to induce hemodynamic compromise and, in some models, cause a paradoxical intracellular acidosis where Carbicarb consistently resulted in intracellular alkalinization (1,2,4). Although major differences in CO_2 production can be demonstrated in experimental animals and humans (16,20), it seemed likely that the different brain and liver pH effects of bicarbonate are mediated by central hemodynamic effects rather than changes in arterial CO_2 tension. In other words, the different effects of these buffers on cardiac function appeared to explain their different systemic effects.

To directly address this issue, the applicant went to the isolated perfused heart preparation to better control experimental variables. Using the isolated heart, we have observed dose dependent decreases in QO_2, dp/dt, along with the metabolic effects of

decreased pHi, [PCr] and increased [Pi] with metabolic acidosis. In these studies, the observed decreases in dp/dt correlated best with increases in [Pim]. Bolus alkalinization of acidotic hearts with bicarbonate which did not affect pHi did not alter cardiac function where alkalinization with the experimental buffer, Carbicarb which did increase pHi also improved cardiac function. A cross-over approach administering drugs in a blinded fashion every twenty minutes was employed in these studies which we have reason to believe, in retrospect, was somewhat flawed (vida infra). These data are contained in reference (8).

To address the relationship between abnormal cardiac energy metabolism and deranged function, comparisons were made between isolated isovolumic hearts exposed to metabolic acidosis and insults which primarily affect energy production, e.g. hypoxia (PaO_2 < 80 torr), substrate depletion (no glucose) and cyanide toxicity (2 mM). Low perfusate calcium (0.6 mM) was used as a model for decreased energy demands. In the hearts subjected to metabolic acidosis, hypoxia, substrate depletion and cyanide but not low perfusate calcium, a strong correlation between [Pim] and dp/dt was observed. Simply put, metabolic acidosis has metabolic consequences similar to those associated with primary energy production failure, and dissimilar to those associated with primary reductions in energy requirements.

We would, therefore, characterize acidosis as inducing a primary decrease in energy production. With metabolic acidosis the associated intracellular acidosis results in greater increases in [Pim] than those resulting simply from the increases in [Pi] from deranged metabolism. This supports our observation that with metabolic acidosis, contractility is more impaired than would be expected by the degree of deranged energy metabolism alone. Therefore, we tentatively concluded that there are, in fact, synergistic effects of pHi and [Pi] on contractile sensitivity to calcium during metabolic acidosis, perhaps mediated through Pim. This point is discussed in some detail in reference (5).

Objective: The objective of the proposed project was to determine the metabolic mechanisms by which cardiac function is depressed during severe acidosis and the pharmacologic maneuvers by which functional recovery may be enhanced or accelerated.

BODY OF THE REPORT:

Experimental Methods:

Animals:

Sprague-Dawley rats (approximately 350 grams body weight) were used in these experiments. Rats were anesthetized with Ketamine (1 ml/kg BW, 50 mg/dl) and Xylazine (0.6 ml/kg BW, 20 mg/ml) prior to surgery. The technique used for removal of the heart was as described by Ross (26) and as we have previously reported (5,8).

Isolated Heart Perfusion:

A Langendorff heart preparation modified for serial P-31 nuclear magnetic resonance spectroscopy (NMR) studies was employed. Circuit design allowed for either maintaining constant perfusion pressure with perfusion flow rate changing in response to changes in coronary vascular resistance or constant perfusion pressure with changes in perfusion pressure occurring with changes in coronary vascular resistance. Perfusate composition (modified Krebs-Henseleit saline without phosphate) and probe construction were as we have previously described in our laboratory (5,8).

The preparation employed was an isovolumic one. The ventricular pressure was monitored continuously using a latex balloon filled to achieve an end-diastolic pressure of 8-12 mmHg at the beginning of each experiment without changing balloon volume for the duration of the experiment as we have also previously reported (5). Blood Gas Determinations: Arterial and venous perfusate were analyzed for pO_2, pCO_2 and pH using a blood-gas instrument (Radiometer Instruments, Copenhagen, Denmark).

Nuclear Magnetic Resonance Spectroscopy:

All NMR spectroscopy studies were performed on a 7.05 Tesla 10 cm bore cryomagnet with AM 300 spectrometer (Bruker Instruments, Billerica, MA).

^{31}P NMR spectroscopy:

^{31}P NMR spectra were obtained serially using three hundred 45 degree pulses (4μs determined on isolated hearts) separated by 1 second relaxation delays using a sweep width of 10000 Hz and 2K data arrays. These arrays were 0 filled to 4K prior to exponential multiplication with 20 Hz linebroadening and Fourier transformation. Signal to noise on all spectra exceeded 50:1. Spectral peak assignments were made on the basis of chemical shift. Because of the small degree of peak overlap, relative peak areas were calculated by a simple integration routine following baseline correction. Corrections for partial saturation of spectral peaks was made as described by other workers. These relative chemical concentrations were converted to absolute concentrations (μmol/g dry) assuming an adenosine triphosphate (ATP) concentration of 16 μmol/g dry at the beginning of perfusion and absolute intensity scaling. An external standard using methylene diphosphate solution was used to control for any changes in coil sensitivity. The intracellular pH (pHi) was estimated based on the chemical shift of the inorganic phosphate (Pi) resonance relative to the creatine phosphate (PCr) resonance (σ) according to the relationship pH = 6.8 + $\log_{10}$ ((σ-3.4)/(5.7-σ)) (5,8).

^{23}Na NMR Spectroscopy:

We used ^{23}Na NMR spectroscopy in an effort to monitor the intracellular sodium concentration. The distinguishing of intracellular from extracellular concentrations of sodium was addressed using chemical shift agents (e.g. 5 mM dysprosium tripolyphosphate or dysprosium TTHA) added to the perfusate for perfused organ studies as we discussed in a recent review (27).

Measurement of Tissue Lactate Concentrations:

Following perfusion, isolated hearts were freeze clamped with Wollenberger clamps cooled in liquid nitrogen and stored at -70 °C until subsequent extraction with perchloric acid (6%). The tissue extract was neutralized to pH 7.0 with KOH and lyophilized to a powder which was resuspended in D_2O spiked with trimethylsilylproprionate (0.25 mM). An ^{1}H NMR spectrum was obtained with a 5 mm commercial probe using 128 90^0 pulses with a relaxation delay of 10 seconds using 8K data files and a sweep width of 4000 Hz. Tissue lactate concentrations were then determined using the relative peak area of the lactate resonance at 1.3 ppm compared with the TSP resonance at 0 ppm correcting for the number of protons contributing to each resonance as we have described previously (28).

Isolated Mitochondria:

Isolated mitochondria were prepared from rat hearts using the method of Chance and Hagihara (29) with minor modifications. Hearts from 2-3 rats were rapidly removed and placed in a 50 ml beaker half filled with chilled isolation solution (75 mM sucrose, 1mM EGTA, 225 mM Mannitol, 2mM Hepes (pH 7.60), and fatty acid free bovine serum albumin fraction V (0.5%), minced and rinsed with this media several times. Nagarse was added (2.5 mg per gm of heart tissue) to approximately 30 ml of isolation solution, and the preparation was homogenized with a Teflon pestle with four up and down strokes. The tissue was then centrifuged at 480g to remove cellular debris, and the supernatant then spun at 7700g for 10 minutes. The pellet which contains isolated mitochondria was removed and the supernatant discarded. The mitochondrial pellet was resuspended in isolation solution (without nagarse). We then aliquoted 0.2 ml of supernatant for a protein determination using biuret method. The mitochondria (1 mg protein/ml) were then studied in incubation solution containing 154 mM KCl, 8 mM MgCl2, 15 mM HEPES and 5 mM KPhos with pH 7.0 at 30°C in a 500 μl stirred chamber with an O2 electrode (Clark). The pH of the incubation

solution was adjusted using HCl or KOH to be either 6.0, 6.5, 7.0, 7.5 or 8.0. Substrates (glutamate and malate [N=20] both 4 mM, succinate [N=7] 4 mM, pyruvate [N=7] 4 mM or octanoate [N=7] 4 mM were added as the potassium salt to the incubation media (with their pH adjusted prior to their addition at the desired final pH) and then ADP (1 μmole) was added with the O2 tension in the chamber being recorded continuously (20 Hz) through an A-D converter into an IBM compatible computer. The rate of oxygen utilization following ADP addition (State 3) and after consumption of the ADP (State 4) as well as the respiratory control ratio (RCR = State 3/State 4) were determined using software in BASIC® written by one of us (JIS). All reagents were obtained from Sigma Chemical Co., St. Louis MO. All studies were run in duplicate with the average value of the two runs considered as one data point.

Statistical Analysis:

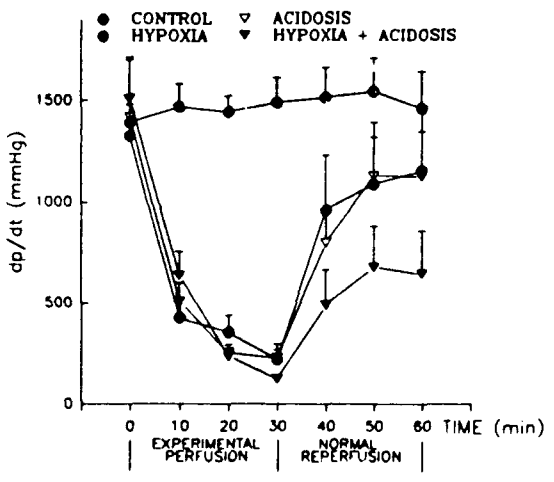
All experiments were performed and the data analyzed with the experimenter blinded to the biological nature of the experiment (e.g. using numbered drug vials). Data obtained before and after experimental manipulations was compared using 1 or 2 way analysis of variance and unpaired or paired t-test with Scheffe's correction for multiple comparisons depending on the unpaired or paired nature or the data. Correlation analysis was performed using simple linear and multi-linear regression methods. Nonparametric analysis was used where appropriate. CRUNCH4® Software will we used as both a spreadsheet and performing statistical analysis (30).

EXPERIMENTAL DATA:

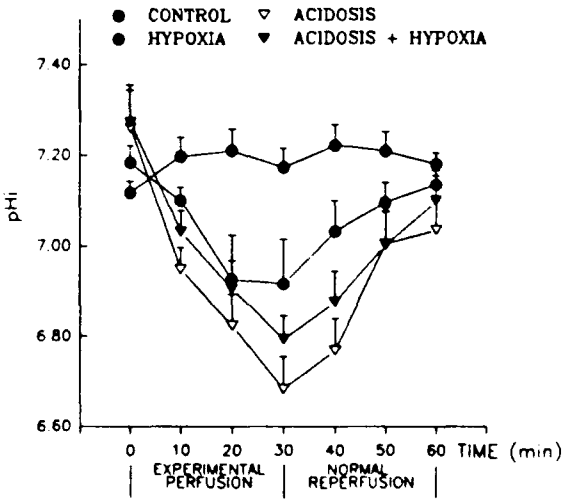
A detailed study of the recovery from acidosis as outlined in protocol 3 of the proposal was performed initially. Because of the importance of hypoxia in the generation of acidosis, interactions between hypoxia and acidosis were also addressed. the following protocol was performed: Isolated hearts were exposed to control perfusion conditions for 30 minutes to establish baseline measurements. They were then subjected to either continued control conditions (C), hypoxia (perfusate PO2 < 60 as compared to typical perfusate PO2 > 400 torr), metabolic acidosis (perfusate pH = 6.8 instead of normal perfusate pH = 7.4) or metabolic acidosis + hypoxia for 30 minutes. Then, all hearts were perfusate under control conditions for an additional 30 minutes of recovery. The results of this study are summarized in the adjacent graphs.

Data are expressed as the mean ± SEM. Statistical comparisons are shown on the accompanying figure legends. This paper has recently been accepted (pending revisions by Magnetic Resonance in Medicine (31). A preprint of this paper may be found in the appendix.

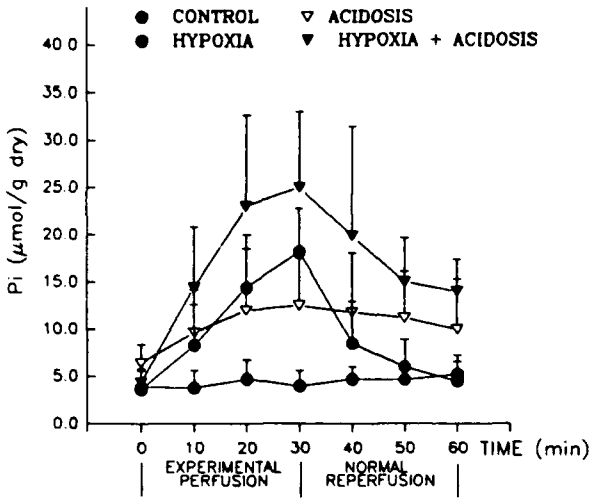
EFFECT OF ACIDOSIS AND HYPOXIA ON dp/dt



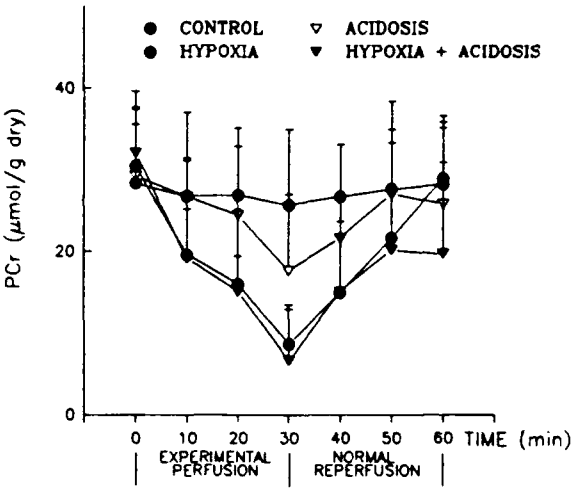
EFFECT OF ACIDOSIS AND HYPOXIA ON pHi



EFFECT OF ACIDOSIS AND HYPOXIA ON TISSUE INORGANIC PHOSPHATE (Pi)

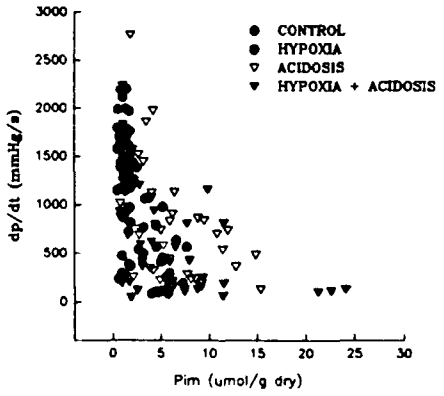


EFFECT OF ACIDOSIS AND HYPOXIA ON TISSUE CREATINE PHOSPHATE (PCr)

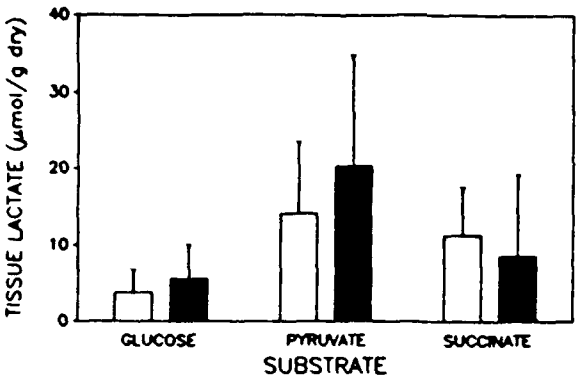


Examining the mechanisms for potential synergism between acidosis and hypoxia, we plotted the relationship between monovalent inorganic phosphate (Pim) and dp/dt at all time points studied in the four experimental groups. A very tight ($r2 = 0.70$) parabolic relationship was observed between these parameters supporting (but not proving) the hypothesis that the monovalent form of inorganic phosphate mediates cardiac muscle "fatigue" during a variety of insults.

RELATIONSHIP BETWEEN TISSUE MONOVALENT INORGANIC PHOSPHATE (Pim) AND dp/dt IN EXPERIMENTAL GROUPS



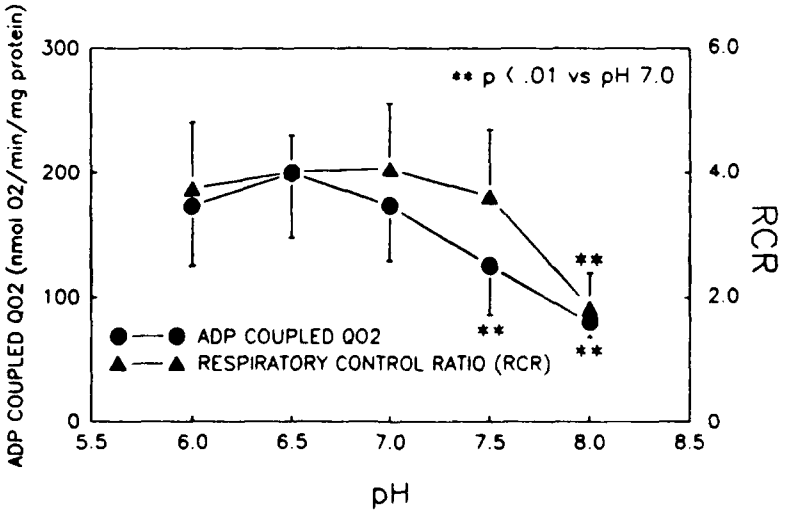
This observed synergism between acidosis and hypoxia in our system supports the notion that acidosis does primarily decrease the isolated heart's ability to produce energy. To address the mechanism behind this deranged energy metabolism, the following studies were performed. First, hearts were perfused with different substrates which either require (glucose) or do not require (pyruvate, succinate and glutamate) glycolytic metabolism. These substrates were used both in the presence of normal or low bicarbonate concentrations. With each of these substrates, induction of acidosis resulted in marked functional and energy metabolic alterations. The effect of acidosis with each of these substrates on functional and metabolic parameters are shown in Table 1 of Reference # 34 which may be found in the accompanying Appendix. Analysis of these data demonstrates that with each substrate, acidosis induces very significant decreases in QO_2, dp/dt and alterations in energy metabolism manifested with each substrate. Most notably, however, was that the effects of acidosis were not different among the different substrate groups. Tissue lactate concentrations at the end of perfusate were not significantly affected by acidosis with any substrate (see figure below) further supporting the idea that the major in vivo site of metabolic blockade with acidosis is not glycolysis. This is discussed in some detail in Reference #34 (see Appendix).



Effect of Acidosis on tissue lactate with different substrates. Open bars: Normal perfusion conditions. Closed bars: Acidosis conditions.

These data suggest that the majority of the effects of acidosis on energy metabolism of the isolated heart result from impaired oxidative phosphorylation rather than an inhibition at the level of glycolytic enzymes. To support this notion, on the evaluation of isolated heart tissue extracts we observed comparable tissue levels of lactate in acidotic as compared to control hearts. Other workers have observed a relative preservation of glycolysis in the isolated heart subjected to respiratory acidosis (32). We should stress, however, that this does not exclude an important inhibitory effect of acidosis on glycolysis under conditions where glycolysis rates would be expected to be increased (e.g. under hypoxic conditions).

To further address this, we performed work in an isolated heart mitochondria preparation subjecting this preparation to different incubation pH. Using a variety of substrates (malate/glutamate N=20, succinate N=7, pyruvate N=7 and octanoate N=7), it was very clear that even severe acidosis (pH = 6.0) did not dramatically impair oxidative phosphorylation by these isolated mitochondria. Some of these data are shown below (data expressed as mean $\pm$ SD, RCR (respiratory control ratio) refers to the ratio of O_2 consumption during state 3 to state 4).



Plot of both ADP coupled respiration (State 3) and respiratory control ratio (RCR) vs pH.

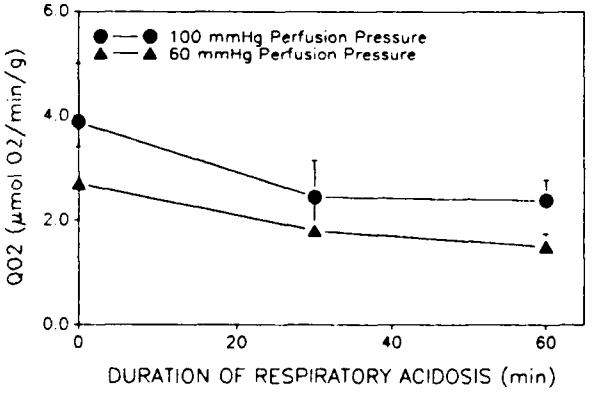
Thus, a direct effect of acidosis on mitochondrial oxidative capacity has essentially been eliminated as a possibility, at least in the heart. Therefore, it appears that

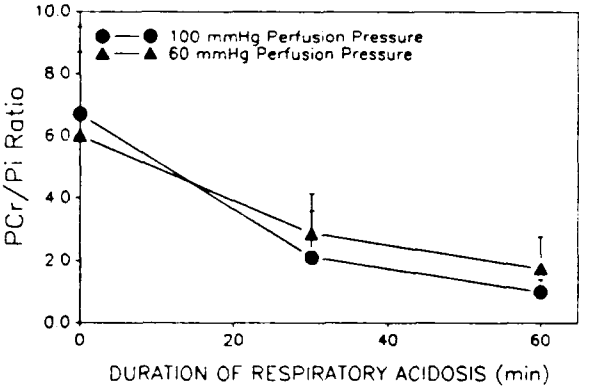
secondary effects of acidosis must mediate the energy production failure at the level of oxidative metabolism.

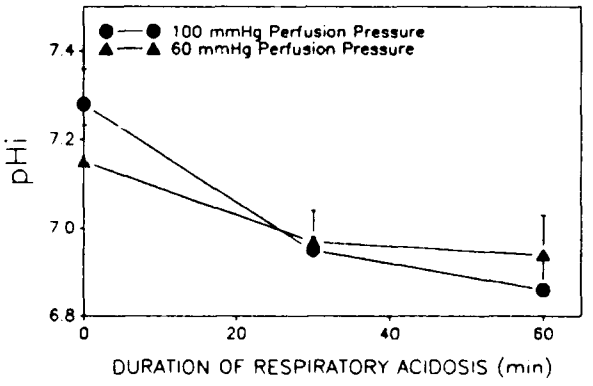
In this project, we also performed a detailed study of the induction of respiratory acidosis outlined in protocol 1b of the proposal. Basically the following protocol was performed:

Isolated hearts were exposed to control perfusion conditions for 30 minutes to establish baseline measurements. They were then subjected to either continued control conditions or respiratory acidosis induced by gassing with 22.5% CO2 balance O2 (as compared to gassing with the usual 7.5% CO2 balance O2) for 60 additional minutes. With this degree of respiratory acidosis, acceptable oxygen delivery rates could be achieved with all PO2 values exceeding 300 torr. Efforts to raise PCO2 to approximately 120 were accompanied by significant relative hypoxia (PO2 < 280) which made interpretation of data difficult. Although these hearts (N=3) showed a rapid and consistent decrease in pHi, dp/dt, [PCr] and QO2 as well as increases in [Pi], the relative hypoxia complicated the interpretation of these data. We ran some relative hypoxia controls (PO2 250-300 torr, N=8) which showed a very unstable time course. Therefore, we restricted our interpretation to that of the moderate hypercapnia group. these data are reported below. Studies were performed at either a perfusion pressure of 100 mmHg or 60 mmHg. Induction of respiratory acidosis with 22.5% CO2 induced an increase in PCO2 to anywhere from 80-100 torr with a decrease in pH to 7.04 ± .05 (mean ± sd). Metabolic effects of respiratory acidosis are shown below at the two perfusion pressures (Both N=8, data expressed as mean ± SD, statistics not shown on the figures).

Control perfusions at these perfusion pressures had stable metabolic parameters during this time frame (data not shown, Both N=8)). Respiratory acidosis resulted in consistent and profound decreases in QO2, the ratio of PCr/Pi and the intracellular pH (all comparisons < p< .01 at 60 minutes, all comparisons except the PCr/Pi ratio p < .01 at 30 minutes with the PCr/Pi ratio comparison p < .05 at this time point).



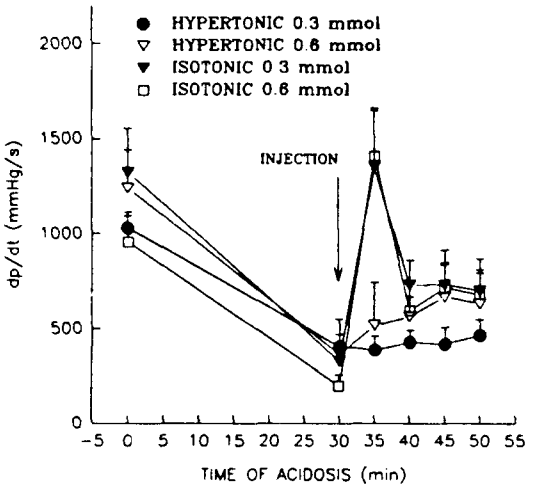




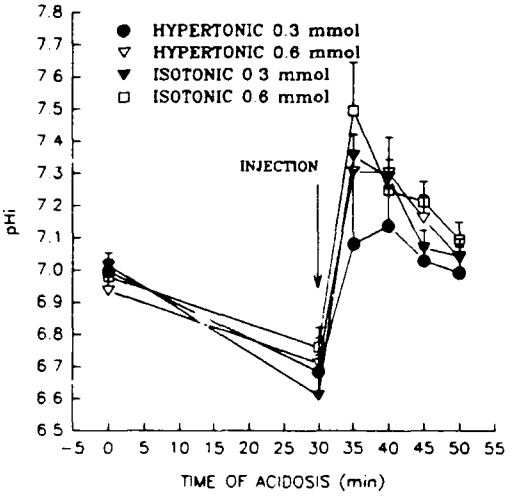
The effectiveness of Carbicarb administration in the setting of metabolic acidosis was evaluated in some detail. Two doses of isotonic (150 mM Na) and hypertonic (1M Na) Carbicarb, (0.3 and 0.6 mmol of Na) injected over 2 minutes were administered to acidotic

hearts (N=8 each group). Carbicarb at both doses and tonicities had considerable alkalinizing effects on pHi (all time points post therapy p< 0.01 vs 30 minutes of acidosis with both doses and tonicities of Carbicarb) as well as resulted in sustained increases in dp/dt (For isotonic Carbicarb, all time points post therapy were p< 0.01 vs 30 minutes of acidosis. For hypertonic Carbicarb, time points 10, 15 and 20 minutes post therapy were statistically different from 30 minutes of acidosis (p < 0.05 for 10 min and p< 0.01 for 15 and 20 minutes) for the higher dose only) and decreases in [Pi] (for both drugs, time points 10, 15 and 20 minutes post therapy were statistically different (p , 0.05 at 20 minutes with 0.3 mmol hypertonic Carbicarb, all other data p < 0.01 vs 30 minutes of acidosis). ATP concentrations were not significantly effected by Carbicarb injection. However, considerable differences in the effects of isotonic and hypertonic Carbicarb on dp/dt and pHi were noted, especially at the earlier (e.g. within 5 minutes) time points. These data are shown below.

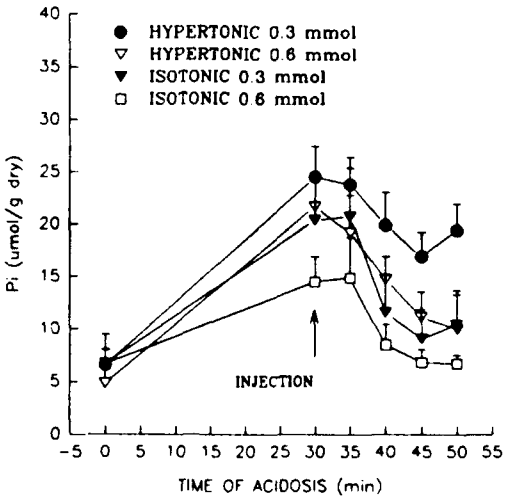
EFFECT OF TONICITY OF CARBICARB ON CONTRACTILE FUNCTION (dp/dt)



EFFECT OF TONICITY OF CARBICARB ON INTRACELLULAR pH (pHi)

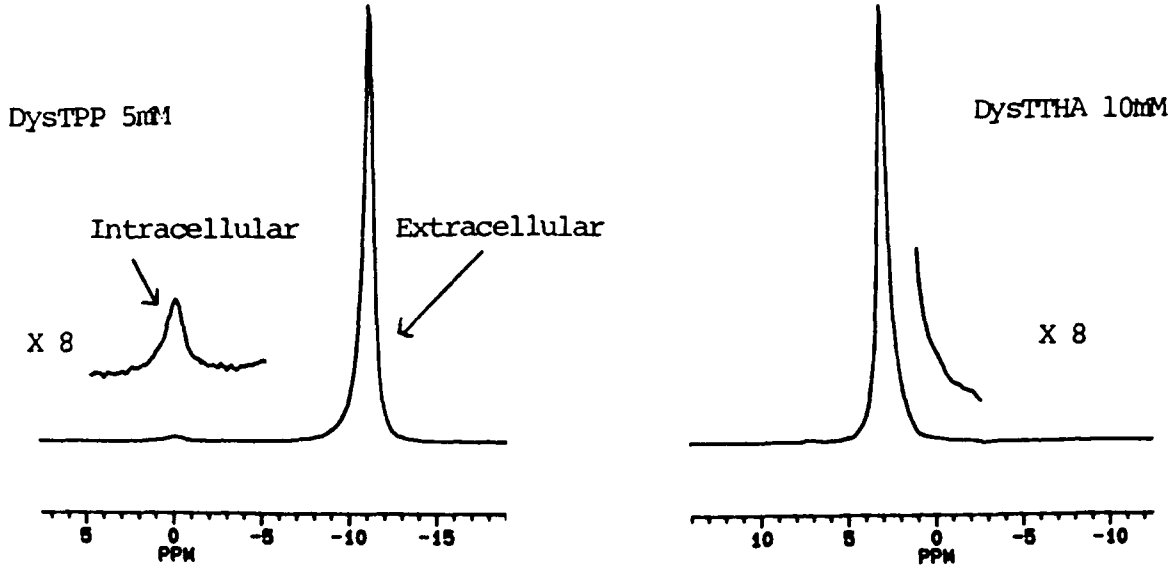


EFFECT OF TONICITY OF CARBICARB ON TISSUE INORGANIC PHOSPHATE (Pi)

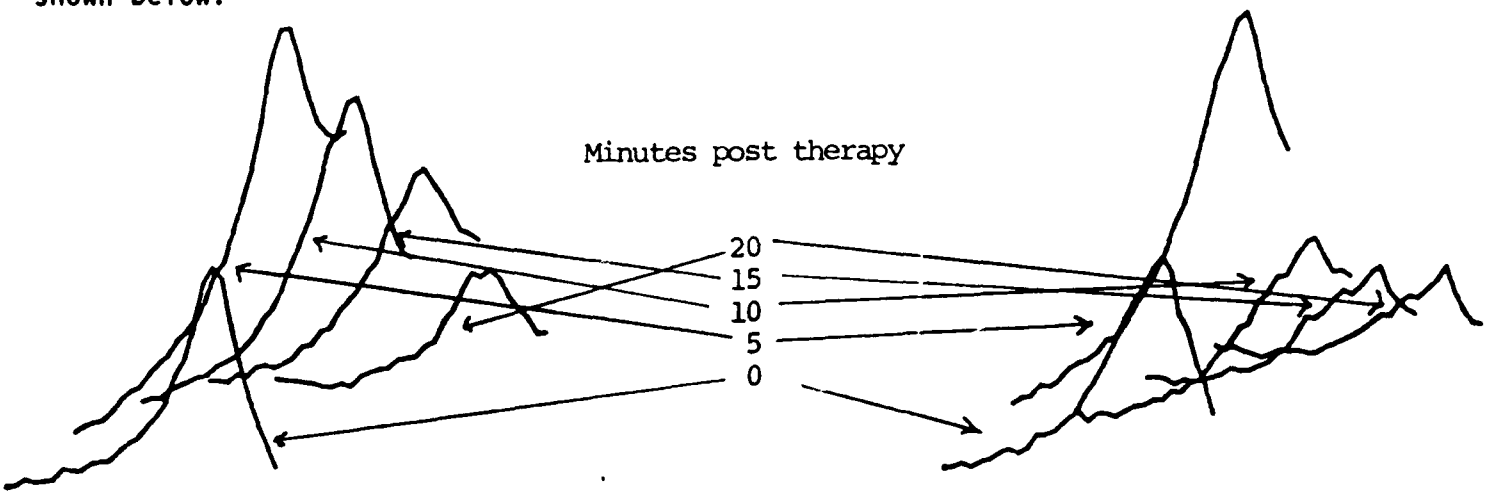


Because of the potential importance of different changes in cytosolic sodium with the isotonic and hypertonic doses of Carbicarb, we have also spent further efforts to examine intracellular sodium concentrations. Our work with dysprosium tripolyphosphate (DysTPP) was disappointing with respect to cardiac function at both 1.2 mM and 2.4 mM calcium

concentrations. However, intracellular sodium concentrations were stable with the 1.2 but not the 2.4 mM calcium concentration. Resolution of intracellular from extracellular sodium was quite simple with DysTPP. A representative spectrum is shown below. Because of these problems with DysTPP, we performed pilot studies (N=8) with DysTTHA (10 mM) as the chemical shift agent as described by Cala and coworkers (33). This agent was relatively well tolerated hemodynamically; however, clear identification of the cytosolic sodium resonance was difficult because of inadequate chemical shift. A representative ^{23}Na spectrum obtained from an isolated heart perfused with 10 mM DysTTHA is shown below.



Because of our failure to resolve the intracellular from extracellular Na resonance with DysTTHA (20 mM), we decided to perform preliminary studies of acidosis and Carbicarb therapy using DysTPP (N=4 with both isotonic and hypertonic Carbicarb, both 0.6 mmol of Na dose). Metabolic acidosis resulted in a 50-100% increase in cytosolic sodium concentration. Marked transient increases in cytosolic sodium occurred with the hypertonic Carbicarb injections whereas consistent decreases in cytosolic sodium occurred with the isotonic Carbicarb injections. Representative stacked plots of the cytosolic sodium peak following induction of acidosis and therapy with isotonic and hypertonic Carbicarb are shown below.



Stacked Plot of intracellular sodium after 30 minutes of acidosis and then 5, 10, 15 and 20 minutes following hypertonic Carbicarb.

Stacked plot of intracellular sodium resonance after 30 minutes of acidosis and 5, 10, 15 and 20 minutes following injection of isotonic Carbicarb.

CONCLUSIONS:

Based on these data as well as our previous experience and a review of the literature, we can conclude that:

1) Energy metabolism is abnormal during metabolic acidosis. Based on the ^{31}P NMR spectral data, inadequate energy production for the existing energy demand appears to result from metabolic acidosis, and synergism with hypoxic cell injury both in terms of function during the period of hypoxia as well as during the recovery period can be demonstrated.

2) This abnormal energy metabolism resulting in higher tissue concentrations of Pi may be involved (along with decreases in pHi) in the deranged cardiac contractile function seen with acidosis.

3) Oxidative metabolism rather that glycolysis appears to be primarily affected by acidosis in our model, although more definitive studies was necessary to confirm this point. Moreover, it is possible that in a setting where glycolysis rates would otherwise be augmented (e.g. under hypoxic conditions), inhibitory effects of acidosis on glycolysis may become more demonstrable.

4) Mitochondrial function does not appear to be <u>directly</u> affected by acidosis. Regarding this last point, it should be stressed that secondary effects on mitochondrial function, e.g. through elevated concentrations of cytosolic sodium, impaired transport of fatty acids into the mitochondria have not been excluded, or altered oxygen delivery resulting from acidosis have not yet been addressed as possible mechanisms by which oxidative metabolism could be <u>secondarily</u> affected during acidosis.

5) Intracellular alkalinization with Carbicarb improves both functional performance as well as energy metabolism in the isolated heart. The sodium concentration of Carbicarb, however, may impact considerably upon the beneficial effects of this agent.

Because of the potential relevance of these data to the USAMRDC mission, continued project support in the form of an extramural grant application has been requested. This application entitled "Cardiac Energy Metabolism During Systemic Acidosis or Hypoxia: therapeutic Implications to Battlefield Injury" (Log # 91248002) and is currently under review.

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APPENDIX

Personnel receiving salary support from this project:

Name	Position	Amount
Nancy Elkins, B.S.	Professional Research Assistant	$5,000.00

Manuscripts resulting from this project at the time of this report:

Suleymanlar,G., H.Z. Zhou, M. McCormack, N. Elkins, R. Kucera, O.K. Reiss, and J.I. Shapiro. Am. J. Physiol. In Press.

Zhou, H.Z., D. Malhotra, J. Doers, and J.I. Shapiro. 1992. Synergism between hypoxic injury and acidosis in the isolated heart. Magn. Reson. Med In press:

Copies of these manuscripts (preprints) are attached in this appendix. Both of these projects have been presented at research meetings. The data in the Suleymanlar et al paper was presented at the American Heart Association Symposium of Clinician Scientists and Established Investigators (1991). The data in the Zhou et al paper was presented at the Western Society for Clinical Research (1992). At least one other manuscript is in preparation which will cite USAMRDC support. Copies of this manuscript will, of course, be forwarded as soon as it is ready for submission. We plan to submit these data also as an abstract for presentation to the 1993 American Heart Association meeting.